AMENDMENTS TO THE SPECIFICATION:

Please amend the specification as follows:

Please amend paragraph 3 to read as follows:

[003] Marine green alga such as *Ulva lactuca* and *Monostroma nitidum* are known to form a ~~callus~~ thallus or become single ~~celled~~ cells as they become purified when grown in defined synthetic media in indoor culture (L. Provasoli; Ulva. Biol. Bull., 1958, 114, 375. : M. Tatewaki, L. Provasoli and I. J. Pintner; J. Phycol., 1983, 19, 409). Moreover, it is also known that when some soil extracts, red alga extracts, brown alga extracts, or certain extracts from marine-derived microorganisms are added to the alga that lost their morphologies in this way, the thallus is formed and the propagation speed increases. (M. Tatewaki, L. Provasoli and I. J. Pintner; J. Phycol., 1983, 19, 409.) However, their active elements and vitamin-like active substances have not been identified, which creates a difficulty in studying their physiology and life or to maintain the culture for a long period using the foliate green alga such as *Ulva lactuca*, and *Monostroma nitidum* under aseptic conditions such as indoor cultivation.

Please amend paragraphs 6 and 7 to read as follows, removing the errant paragraph break between them, and adjust the numbering of the subsequent paragraphs accordingly.

[006] In view of the foregoing situations, the inventor of the present invention investigated novel active components in order to isolate the active substances having strong activity from the culture solutions of YM-2-23 strain (Deposited to International Patent Organism Depository, National Institute of Advanced Industrial Science and Technology (Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, Japan) with deposition number

FERM BP-8417 on August 20, 2001 (original depository) (Transfer request accepted to the original depository on June 25, 2003 under the Budapest Treaty)), *Tenacibaculum* sp., YH-1-69 (Deposited to International Patent Organism Depository, National Institute of Advanced Industrial Science and Technology (Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, Japan) with deposition number FERM BP-8418 on August 20, 2001 (original depository) (Transfer request accepted to the original depository on June 25, 2003 under the Budapest Treaty)), and their analogous strains, to discover if these compounds are novel substances to complete the present invention. Previously, for substances produced by ~~Flavobacterium, Zobellia or Tenacibaculum~~ *Flavobacterium, Zobellia,* or *Tenacibaculum* analogous to YM-2-23 strain and/or YM-1-69 strain, the carotenoid derivatives such as lycopene and zeaxanthin are reported, but there have been no cases of isolated chemical substances with foliate alga promoting activity or the morphogenetic control activity, and this will be the first such report in the world.

Please amend paragraph 24 to read as follows:

[024] The novel chemical substances 1 and 2 of the present invention can be produced using microorganisms. The microorganisms used for the chemical substance production of the present invention are not limited to the microorganisms exhibiting the ability to produce the chemical substance, and these include, for instance, strains that belong to the *Cytophaga-Flavobacterium- Bacteriodes* ~~comp1ex~~ complex, such as *Flavobacterium*, *Zobellia*, and *Tenacibaculum* and the mutant strains derived from these strains. More specifically, they include the YM-2-23 strain (FERM BP-8417), *Tenacibaculum* sp. YM-1-69 (FERM BP-8418), and the mutant strains derived from these strains. Instead of using the YM-1-69 strain and YM-2-23 strain, analogous

strains ~~of these strains~~ may be used. The "analogous strains of YM-1-69 strain" include, for instance, strains exhibiting thallus forming activity or growth promoting activity against marine foliate green alga as well as the strains having higher than 85%, or higher than 95%, homology to the nucleotide sequence of the 16S rRNA V3 region gene described in Sequence 1 or the strain having higher than 72%, or higher than 95% homology to the nucleotide sequence of the gyr B gene described in Sequence 2. The "analogous strains of YM-2-23 strain" include, for instance, the strains exhibiting the thallus forming activity or growth promoting activity against marine foliate green alga as well as the strains having higher than 85%, or higher than 95%, homology to the nucleotide sequence of the 16S rRNA V3 region gene described in Sequence 3 or the strain having higher than 72%, higher than 80%, or higher than 95%, homology to the nucleotide sequence of the gyr B gene described in Sequence 4.

Please amend paragraph 25 to read as follows:

[025] The "YM-1-69 strain analogous strain" and the "YM-2-23 strain analogous strain" includes ~~YM-2-10~~ YM2-10 (MBIC 04671), YM2-11 (MBIC 04672), YM2-12 (MBIC 04673), YM2-13 (MBIC 04674), YM1-66 (MBIC 04663), YM2-24 (MBIC 04684), ~~YMI-51~~ YM1-51 (MBIC 04662), ~~Zobellia uliginosa~~ *Zobellia uliginosa* (ATCC 14397), YM1-11 (MBIC 04693), T-588 (MBIC 05930), YM2-22 (MBIC 04682), YM2-27 (MBIC 04687), YM2-6 (MBIC 04669), ~~YMI-68~~ YM1-68 (MBIC 04664), ~~YMI-38~~ YM1-38 (MBIC 04661), YM2-4 MBIC 04667), YM2-5 (MBIC 04668), YM2-7 (MBIC 04670), YM2-21 (MBIC 04681),YM2-1 (MBIC 04666), T-565 (MBIC 05877), T-424 (MBIC 05876), [*Cytophaga*] sp. UP7 (MBIC 01484), T-551 (MBIC 05929), *Pedobacter heparinus* (IFO 12017), T-561

(MBIC 05879), *Cyclobacterium marinum* (LMG 13164), ~~Cytophaga~~ *Cytophaga* sp. (MBIC 01539), *Cytophaga* sp. (MBIC 01599), and *Chitinophaga pinensis* (DSM 2588).

Please amend paragraph 26 to read as follows:

[026] Among said strains, strains having **[[']]**"MBIC" in their names are available from the Marine Biotechnology Institute Culture Collection (MBIC) (3-75-1 ~~Hirata~~ Heita Kamaishi-city, Iwate, Japan) (http://seasquirt.mbio.co.jp/mbic/index.php?page=top). Strains having "IFO" in their names are available from the Institute for Fermentation, Osaka (IFO) (17-85 2-chome Honmachi Juso Yodogawa-ku, Osaka, Osaka, Japan), strains having "ATCC" in their names are available from American Type Culture Collection (ATCC) (12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A.), strains having "DSM" in their name are available from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) (Mascheroder Weg 1b, 38124 Braunscheig, Germany), and strains having "LMG" in their name are available from BCCM™/LMG Bateria Collection (Belgian Co-ordinated Collections of Micro-organisms, Laboratorium voor Microbiologie, Universiteit Gent (RUG), K. L. Ledegancksfaat 35, B-9000 Gent, Brussels, Belgium).

Please amend paragraph 32 to read as follows:

[032] In order to culture the novel chemical substance 1 or 2 in the bacteria, liquid culture is the most efficient, a temperature of approximately 30°C is appropriate, and the pH of the medium is normally between 7 to 9, or 7.5 to 8. Sodium hydroxide solution or hydrochloric acid may be used for pH adjustment of the culturing media.

Please amend paragraph 33 to read as follows:

[033] The novel chemical ~~substance~~ substances 1 **[[**or**]]** and 2 are produced in the media and in the bacterial cells 1 to 4 **[[**hours**]]** days after incubating the bacterial cells in the media. One may terminate the cultivation when the substance production in the medium reaches its maximum. More specifically, 3-day incubation may be used.

Please amend paragraph 35 to read as follows:

[035] The treatment of the novel chemical substance 1 with appropriate methylating agents (for instance, trimethylsilyldiazomethane) produces the mono-, di-, and tri-methyl forms of the novel chemical substance 1. The **[[**mol**]]** molar ratio of trimethylsilyldiazomethane to the novel chemical substance 1 can be adjusted, and appropriate reaction conditions (for instance, reaction temperature, pH setting, or reaction with the presence of diethylaminosulphur trifluoride (DAST)) to selectively obtain mono-, di-, and tri-methyl forms thereof) can be selected.

Please amend paragraph 36 to read as follows:

[036] Furthermore, by treating **[[**said**]]** tri-methyl form with **[[**said**]]** sodium borohydride, ~~Mel1H3~~ Me1H3 with the physiochemical properties**[[**,**]]** described below**[[**,**]]** is obtained. In addition, by changing the polarity of the reaction solvent and reaction speed, a further reduced chemical substance from Me1H3, namely Me1H1, is obtained. Furthermore, by treating Me1H1 with alkyl iodide (for instance, methyl iodide) shown as formula: RI (here R indicates the alkyl group with carbon number 1 to 6 under strong alkali condition, alkyl forms, such as the methylated form Me1H1Me is obtained). These reactions and the information on the product obtained are useful in identifying the novel chemical substances 1 and 2.

Please amend paragraph 37 to read as follows:

[037] Furthermore, by treating Me1H1, obtained by trimethylating the novel chemical substance 1 and treating with sodium borohydride, with hydrochloric acid–methanol for a short period or hydrated methanol for a long period, a cyclized form Me1H1W4 is obtained. Me1H1W4 is easily crystallized in various organic solvents (for instance, acetone, diethyl ether, hexane and dichloromethane chloroform), and its structure can be determined with X-ray crystallographic analysis. Based on the structure of ~~Me1HlW4~~ Me1H1W4, the structure of the novel chemical substance 1 can be determined by tracing back the reaction processes of the derivatives.

Please amend paragraph 39 to read as follows:

[039] Marine foliate green alga is ideal for the alga being cultivated in **[[**said**]]** culture medium for alga. Seaweed in Ulvales, green alga Monostromataceae and Ulvaceae, for instance are included for the marine foliate green alga. More specifically, these green algae include *Monostroma nitidum*, *Monostroma oxyspermum* and *Monostroma angicava* for Monostromataceae, and *Enteromorpha compressa*, *Enteromorpha intestinalis*, *Enteromorpha linza*, *Ulva conglobata*, and *Ulva perusa* for Ulvaceae.

Please amend paragraph 64 to read as follows:

[064] The strains used for the present invention were isolated by the procedures described below. Approximately 1 gram of freshly collected alga was added in 10 ml of sterile sea water and then vortexed vigorously for approximately 1 minute. The supernatant was serially diluted further with sterile sea water to 1/10 and 1/100, and 100 µl was inoculated onto 1/10 marine agar plates and then spread to the entire plate with

a sterilized Conradi stick. After culturing for 2-3 days at room temperature, grown yellow-red colonies were individually inoculated in marine agar plates and kept in culture until **[[**the**]]** a single colony was isolated. These cells were transferred into the wells of either 24- or 48-well microtitre plates containing 2 or 1 ml, respectively, of an ASP7 medium, and then approximately 20 cells of single celled *Monostroma oxyspermum* were added. Each single colony from the isolated strains was directly inoculated to two wells using a sterile ~~platinum~~ inoculation loop. The microtitre plates were incubated at 19-22°C with a 14h/10h light/dark cycle for 5 days, and the thallus formation of *Monostroma oxyspermum* was confirmed under an inverted microscope. Strains exhibiting the thallus formation were confirmed by re-examination in a similar manner to that described above. After these screening steps described above, the YM-1-69 strain and YM-2-23 strain were isolated as the strains exhibiting the thallus forming activity. The YM-1-69 strain was isolated from *Halimeda opuntia* (Codiales, Chlorophyta) and the YM-2-23 strain was isolated from *Monostroma nitidum* (Ulvales, Chlorophyta).

Please amend paragraphs 65 and 66 to read as follows, removing the errant paragraph break between them, and adjust the numbering of the subsequent paragraphs accordingly.

[065] The DNA sequences of the 16S rRNA V3 regions and gyr B genes of the microorganisms obtained in Referential Example 1 were determined. The DNA sequences of the 16S rRNA V3 region and gyr B gene of YM-1-69 strain are shown in Sequence 1 and Sequence 2, respectively. In addition, the DNA sequences of the 16S rRNA V3 region and gyr B gene of YM-2-23 strain are shown in Sequence 3 and

Sequence 4, respectively. The obtained DNA sequences were analyzed using the searching database (DDBJ-fasta) and the sequences were shown to have high homology to the microorganisms shown in Table 1.

Please amend paragraph 67 to read as follows:

[067] In addition, Table 2 shows physiochemical properties of the YM-1-69 strain and the YM-2-23 strain.

Table 2

Physiochemical Properties	YM-1-69	YM-2-23
[[Gam]] Gram Staining	-	-
Catalase Activity	+	+
Oxidase Activity	+	+
OF Test	o	F
Mg or Ca Requirements	+	+
Nitrate Reduction	+	+
Indole Production	-	-
Gelatin Hydrolysis	-	-
Starch Hydrolysis	+	+
DNA Hydrolysis	+	+
Tween80 Hydrolysis	+	+
Esculin Hydrolysis	+	+
Arginine Dihydrolase Activity	-	-
Urease Activity	-	-
β-galactosidase activity	-	+
Utilization of Citric Acid Simons Medium	-	-
Christensen Medium	-	-

+:Positive, -: Negative

Please amend paragraph 72 to read as follows:

[072] In addition, the composition of the ASP7 medium is as follows.

Table 3
Modified ASP7 Medium (1L, pH 7.8-8.0)

Distilled Water	950 ml
NaCl	25 g
$MgSO_4 \cdot 7H_2O$	9 g
KCl	700 mg
$CaCl_2$	840 mg
Tris HCl	1 g

[[NaC_3]]	
$NaNO_3$	50 mg
~~Na_3-glyceroPO_4~~	
Na_2-glyceroPO_4	20 mg
~~$Na_2SiO_{39}H_2O$~~	
$Na_2SiO_3 \cdot 9H_2O$	70 mg
Vitamin B12	1 µg
Nitrilotriacetic Acid	70 mg
Vitamin Mix S3 *1	10 ml
PII metals *2	30 ml
S2 metals *3	5 ml

Table 4
*1 Vitamin Mix S3

Distilled Water	100 ml
Thiamin Hydrochloride	5 mg
Nicotinic Acid	1 mg
Calcium Pantothenate	1 mg
p-Aminobenzoic Acid	0.1 mg
Biotin	0.01 mg
Inositol	50 mg
Thymine	30 mg
Folic Acid	0.02 mg

Table 5
*2 PII metals

Distilled Water	1000 ml
Na_2-EDTA	1 g
$FeCl_3 \cdot 6H_2O$	48 mg
H_3BO_3	1.13 g
$MnCl_3$ $4H_2O$	144 mg
$ZnCl_2$	5.2 mg
$CoCl_2 \cdot 6H_2O$	4 mg

Table 6
*3 S2 metals

Distilled Water	500 ml
$Na_2MoO_4 \cdot 2H_2O$	63 mg
NaBr	640 g
$SrCl_2 \cdot 6H_2O$	304 mg
RbCl	14 mg
LiCl	61 mg
KI	0.65 mg
V_2O_5	0.18 mg

[Example 2] Isolation and Purification of the Novel Chemical Substance 1

Please amend paragraph 73 to read as follows:

[073] YM-2-23 strain (FERM BP-8417) was used as a seed bacterium. For main culture medium, marine broth (Difco, 37.4 **[[**g/1**]]** g/l, or prepared by mixing each reagent in accordance with the displayed components). The corresponding strain was cultivated in 50 ml of marine broth in a 100 ml conical flask on a reciprocal shaker (100 rpm) at 30°C for 24 hours, and then the entire medium was transferred in a 1 liter conical flask with a baffle containing 450 ml of the medium to incubate for another 24 hours under the same condition. The main culture was carried out in 800 ml of the medium in 16 units of a 1 liter conical flask with a baffle containing 800 ml of the medium each on a reciprocal shaker (130 rpm) and then further incubated in a 10 units of 1 liter conical flask containing 450 ml of the medium on a reciprocal shaker (100 rpm) at 30°C for 3 days. A total of approximately 18 liters of the culture media obtained in this way was centrifuged. The bacteria cells were stored in a -20°C freezer and the filtered culture media was stored at 4°C until use. The bacteria cells of 4 batches of said main culture (approximately 72 liters) were ~~eluted~~ extracted twice with 1200 ml of 50% acetonitrile solution, and then concentrated. The concentrated solution and approximately 72 liters of the filtered culture media were mixed and then adsorbed by 2500 ml of the styrene-divinyl benzene polymer Diaion HP-20 (Mitsubishi Chemicals). The resin was washed with **[[**600**]]** 6000 ml of 10% acetonitrile solution to demineralize, and then eluted with **[[**600**]]** 6000 ml of 50% acetonitrile solution to obtain a thallus formation inducing fraction. The eluted fraction was adsorbed by ~~Toyota~~ Toyo-Pearl DEAE-650(M) ~~(Tohso)~~ (Tosoh), and then washed with 180 mM NaCl-20% acetonitrile,

followed by elution with 450 mM NaCl-20% acetonitrile to obtain the active fraction. After concentrating the eluted fraction under reduced pressure to eliminate acetonitrile, the fraction was adsorbed with 500 ml of Diaion HP-20 (Mitsubishi Chemicals) and washed with 1000 ml of 10% acetonitrile solution to demineralize, to obtain the active fraction with 1000 ml of 50% acetonitrile solution. After concentration under reduced pressure, the active fraction was freeze-dried to obtain a thallus formation inducing fraction. **[[**Said**]]** The culture and two batches of ~~coarse~~ crude fraction (culture medium approximately 140 liters) were mixed and purified by gel filtration chromatography (Amersham Biosciences, Sephacryl S-100 HR 25 mm (inner diameter) x 1200 mm (length)) using 100 mM NaCl-20 mM Na_2HPO_4-20% acetonitrile solution (pH 9) as a mobile phase to obtain the fraction with thallus inducing activity. This active fraction was concentrated, demineralized, freeze-dried, and isolated with high performance liquid chromatography (Amersham Biosciences, RESOURCE RPC3ML, 6.4 mm (inner diameter) x 100 mm (length) x 2 in series) using 14 to 22% acetonitrile-5 g $(NH_4)2CO_3$/l solution as a mobile phase. Then, the active fraction was freeze-dried, and further purified with high performance liquid chromatography (Amersham Biosciences, RESOURCE RPC3ML, 6.4 mm (inner diameter) x 100mm (length) x 2 in series) using 5-25% acetonitrile - 1% NH_3 as a mobile phase to finally obtain approximately 140 μg of the novel chemical substance 1 with said physiochemical properties of the present invention.

Please amend paragraph 76 to read as follows:

[076] The **[[**thus**]]** obtained compounds had physicochemical properties as shown below.

Please amend paragraph 126 to read as follows:

[126] 1 μg/ml x 10^{-12} = 1 ag/ml (~~atto-gram~~ attogram per milliliter)

Please amend paragraph 127 to read as follows:

[127] As the cultivating period becomes longer, the novel chemical substance 1 is used up by the *Monostroma* grown in the medium, and the thallus will be ~~destroyed~~ collapsed. FIG. 13 shows a picture of a seven-step dilution (final concentration of the novel chemical substance 1: 1 x 10^{-7} μg/ml) cultivated for 10 days. Although traces of thallus are recognized, the disrupted thallus is observed. FIG. 14 shows a picture of a six-step dilution (final concentration of the novel chemical substance 1: 1 x 10^{-6} μg/ml) cultivated for 10 days. The thallus was maintained without being ~~destroyed~~ collapsed, and MEC after 10 days cultivation was as follows.

Please amend paragraph 128 to read as follows:

[128] 1 μg/ml x 10^{-6} **[[**μg/ml**]]** = 1 pg/ml (~~pico-gram~~ picogram per milliliter).

Please amend paragraph 132 to read as follows:

[132] ~~The free-living cells~~ Spores obtained from the *Ulva pertusa* and *Enteromorpha intestinalis* collected from Miho Shimizu-city, Shizuoka were washed using their phototaxis activity in the ASP7 medium supplemented with the antibiotics, and then were transferred to rectangular petri dishes filled with sterilized cover glasses and aseptized for 5 days. When the free-living cells had attached to the cover glasses and had start growing, each cover glass was placed in each hole of 6-MTP, followed by adding 10 ml of the ASP7 medium not supplemented with antibiotics. For a test group, the novel chemical substance 1 was added to result in 1 ng/ml and for the control group, nothing was added. They were incubated for 7 days under the same conditions as in

Example 1. On day 10, the cover glasses of the test group and control section were transferred to the culture test tube containing the medium prepared under the same condition to further incubate another 7 days. FIG. 15 and 16 shows the test results of *Ulva pertusa* and *Enteromorpha intestinalis*, respectively. As seen clearly by comparing the test and control groups, rhizoids of the green alga were abnormally developed and the regular thallus formation was not observed in the control group, but normal development and thallus formation were observed when the novel chemical substance 1 was added. In addition, the ASP7 medium supplemented with antibiotics is an ASP medium, having the following composition and containing 2% antibiotics.

Table 8

Antibiotic Mixture

Distilled Water	1000 ml
Penicillin	100 mg
Streptomycin	200 mg
Kanamycin	100 mg

[Example 8] Specific Activity of Morphogenesis-Inducing Activity Against Foliate Green Alga

Please amend paragraph 133 to read as follows:

[133] Similar to Example 5, the MEC were determined for the YM-1-69 strain, the YM-2-23 strain, and the analogous strains shown in FIG. 17 and shown in FIG. 18. In this figure, the Y-axis indicates the amount of *Monostroma oxyspermum* (ml) can be activated by the addition of 1 μl of the supernatant of the culture media. For instance, YM-2-23 strain is capable of activating approximately **[[800]]** 8000 ml of *Monostroma oxyspermum* with 1 **[[ml]]** μl of the supernatant of the culture media in accordance with FIG. 18.

[Example 9] Preparation and Physiochemical Properties of Me1H1W4 Cyclized Form

Please amend paragraph 135 to read as follows:

[135] [Physiochemical Properties of ~~Me1HlW4~~ Me1H1W4]

Please amend paragraph 140 to read as follows:

[140] Mass Spectroscopy :CIMS: ~~m/z416[M+Hl]+~~ m/z 416 $[M+H]^+$

Please amend paragraph 146 to read as follows:

[146] Purified Me1H1W4 cyclized form can be easily crystallized in an appropriate solvent system, monocyclinic single crystal was obtained when it was re-crystallized with diethyl ether-acetone. The crystal was analyzed with X-ray crystallography, and its ortep drawing is shown in FIG. 21. Moreover, Table 9 shows the data of the crystal. Based on this ortep diagram, the structure of Me1H1W4 is as in Formula 1:

Please amend paragraph 149 to remove the paragraph break shown by "[149]" and include the following test as part of paragraph [148].

~~[149]~~, respectively. The structural formula for the novel chemical substance 1 is shown in Formula 4:

Table 4

Crystal data	
	~~V=4585.6 (4)Å3~~ V=4585.6 (4)$Å^3$
$C_{25}H_{37}NO_4$	Z = 8
$C_{25}H_{37}NO_4$	D_x = 1.204 Mg m^{-3}
M_r = 415.574	Density measured by: not measured
Monoclinic	fine-focus sealed tube
C2	Mo Kα radiation
a = 33.103 (2)Å	λ = 0.71073
b = 9.1120 (4)Å	μ = 0.080 mm-1
c = 15.3240 (10)Å	T = 298 K
α= 90.00°	Cube

β = 97.217 (2)°

γ = 90.00°

Colourless

Crystal source: Local laboratory

Data collection

DIP Image plate

Absorption correction: sphere
T_{min} = 0.959 , T_{max} = 0.959
7315 measured reflections
7315 independent reflections
6796 observed reflections

Criterion : >2sigma(I)
θ_{max} = 25.72 °
h = -39→39
k = -10→10
l = -18→18

Refinement

Refinement on F^2
full matrix lease squares refinement
R(all) = 0.0734
R(gt) = 0.0696
wR(ref) = 0.2123
wR(gt) = 0.2064
S(ref) = 1.073

7315 reflections
559 parameters

1 restraints

Only coordinates of H atoms refined
Calculated weight calc
Δ/σmax = 0.066
$\Delta\rho$max = 0.254eÅ^3
$\Delta\rho$min = ~~-0.560eÅ3~~ -0.560eÅ^3
Extinction correction: none
Atomic scattering factors from
International Tables Vol C
Tables 4.2.6.8 and 6.1.1.4
Flack parameter = 1.9 (15)
Flack H D (1983), Acta Cryst. A39, 876-881

Data collection: DIP Image plate
Data reduction: maXus (Mackay et al., 1999)
Program(s) used to refine structure: SHELXL-97 (Sheldrick, 1997)